EFFECT OF ALKALINE POLYPEPTIDE-CYTOMEDINS FROM KIDNEY TISSUE ON IMMUNITY, HEMOSTASIS, AND THE COURSE OF NEPHRITIS

B. I. Ivanov, N. N. Tsybikov,
N. A. Kolbina, and A. M. Yur'ev

UDC 612.017.1+612.115].06:612.46.
018:577.175.85+616.611-002-092:
616.61-008.6:577.175.85

KEY WORDS: cytomedins, hog kidney, nephritis, immunity.

Alkaline polypeptide cytomedins were first isolated from the animal thymus by Morozov and Khavinson in 1974 [3]. In subsequent years cytomedins have been obtained from other organs: lungs, liver, lymph nodes, brain, skin, and so on. Their physicochemical and biological properties have been studied. All cytomedins have been shown to be polypeptides of basic character with mol. wt. of 2-10 kD, and capable of regulating the functional state of the organ from which they were isolated. The basic mechanism of action of cytomedins on target cells is through the cyclic nucleotide system [3, 5]. Most cytomedins from peripheral organs can exert their effect on homeostasis of the body as a whole, which is realized through their action on the system of immunity and hemostasis [2]. However, cytomedins from renal tissue have not hitherto been studied, and that was accordingly the aim of the present investigation.

EXPERIMENTAL METHOD

Alkaline polypeptides were isolated from hog kidneys by the method of acetic acid extraction [3], and the product was conventionally called "renalin." The preparation was added in doses of 100, 50, 10, and 1 μg to 1 ml of normal human blood plasma and incubated for 15 min at 37°C, after which the dose-dependent change in coagulation of the plasma was investigated by measuring the recalcification, cephalin, kaolin, and prothrombin times, the activated partial thromboplastin time (APTT), and the thrombin and prothrombin times [1].

The immunomodulating properties of renalin were studied in a culture of human peripheral blood lymphocytes from patients with secondary immunodeficiencies (burns of the II-III degree), with determination of receptor expression on T and B lymphocytes by the method in [4]. Rosette-forming cells were counted after incubation of the preparation (10 μg in 1 ml of culture) for 120 min. Thymalin (cytomedins from the thymus) and also renalin, inactivated beforehand at 100°C for 5 min, were used as the control. A lymphocyte attached to at least three erythrocytes was taken to be one rosette-forming cell. Finally, rabbit antirenal serum [6] was injected into 26 rats, and 5 days after the injection, renalin was injected into 13 of the rats in a dose of 50 μg/kg body weight daily for 5 days. The control rats, which had developed Masugi nephritis, were given injections of physiological saline. Blood was taken from the abdominal aorta under ether anesthesia 40 days after injection of the antiserum, stabilized with sodium citrate, and then used for determination of the various parameters of coagulation and fibrinolysis, as well as oxygen consumption, in renal tissue homogenates containing ATP, ADP, and AMP, in peripheral blood. In the other group consisting of 25 animals with Masugi nephritis, treated or not treated with renalin (10th day after development of nephritis), the kidneys were removed from the rats and fixed in 10% neutral foralin solution. Histological celloidin sections were stained with hematoxylin and eosin.

EXPERIMENTAL RESULTS

In the experiments in vitro the preparation lengthened the recalcification time of citrated plasma and increased the prothrombin and cephalin times (Table 1). Renalin exhibited antiprotease properties, which are characteristic of all alkaline polypeptides.

Department of Biochemistry, Department of Pathological Physiology, and Department of Pathological Anatomy, Chita Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 1, pp. 19-21, January, 1989. Original article submitted February 20, 1988.

0007-4888/89/0001-0021$12.50

TABLE 1. Effect of Renalin on Hemostasis and Fibrinolysis (M ± m)

Parameter		Control	Dose of renalin, μg				
			100	50	10	1	0,1
Recalcification time	sec	90,75±5,33	124,33±5,01*	109,25±4,12*	95,66±4,58	106,08±4,58*	99,25±4,56
Prothrombin time		58,83±2,99	73,83±1,58*	68,41±1,16*	64,33±2,29	63,08±2,09	67,62±1,58*
Thrombin time		34,25±1,22	35,58±1,41	34,58±0,82	34,41±1,28	33,75±1,21	34,62±1,9
APTT		66,39±4,14	67,41±4,03	65,63±4,33	66,9±3,78	66,09±3,85	75,0±3,5*
Cephalin time		74,16±4,02	84,08±4,84	78,75±2,55	79,33±2,32	77,58±3,17	77,25±3,58
Fibrinolysis		202±16	216±16	206±16	205±18	198±15	206±18

Legend. Here and in Tables 2 and 3: *p < 0.05.

TABLE 2. Effect of Renalin on Receptor Expression (M ± m)

Parameter,%	Control	Thymalin	Inactivated renalin	Renalin
T-active	21,2±1,9	30,5±3,2*	25,0±2,4	32,0±2,6*
T-total	38,9±2,6	45,4±3,1	43,2±3,1	46,2±4,2
B lymphocytes	42,2±3,0	46,0±3,6	41,3±2,2	47,8±3,0

TABLE 3. Effect of Renalin on Hemostasis and Energy Metabolism (M ± m)

Parameter		Control	Expt. 1	Expt. 2
Recalcification time	sec	63,3±1,5	63,25±3,1	63,4±4,1
Thrombin time		28,5±0,6	27,1±0,6	27,1±0,9
Prothrombin time		30,3±1,6	40,2±1,5*	42,2±1,6*
APTT		40,1±1,0	27,2±1,1*	25,1±1,4*
Cephalin time		39,6±1,2	38,5±1,3	36,5±1,5
Euglobulin fibrinolysis time		224,5±6,2	244,4±4,3*	214,0±5,2
O_2 consumption, μl/mg tissue		6,6±0,7	4,5±0,9*	6,1±0,4
ATP	μmoles/m	149,0±3,0	200,2±2,8*	125,6±1,3*
ADP		63,5±2,9	62,9±2,3	47,5±1,7*
AMP		37,1±1,02	38,9±1,3	29,8±0,7*

Legend. Expt. 1) Animals with Masugi nephritis, not treated with renalin; Expt. 2) animals with Masugi nephritis, treated with renalin.

The number of T-active lymphocytes after incubation with renalin showed a statistically significant increase. The total number of T lymphocytes and B lymphocytes was increased, although this is a statistically probable shift (Table 2). Experiments in vivo showed that in rats with Masugi nephritis the prothrombin time was lengthened, the activated partial prothrombin time was shortened, and fibrinolysis was inhibited. The basic parameters of the coagulogram remained substantially unchanged. In rats treated with renalin, euglobulin fibrinolysis was activated by comparison with the untreated animals, but APTT remained shortened. Parallel with the changes observed in the rats with Masugi nephritis the level of tissue respiration of the kidneys was lowered. Meanwhile the ATP concentration in the blood was raised and the ADP and AMP levels unchanged. In rats receiving renalin the tissue respiration level was close to values obtained in intact animals, although the ATP, ADP, and AMP concentrations remained low.

In the course of the experiment 50% of the untreated rats died, compared with only 9% of the intact rats and 13% of those treated with renalin. The untreated rats were disinclined to move, they lost their hair, and their water intake was increased (Table 3). On histologi-

cal investigation the morphological pattern of the renal lesion in the experimental group differed from the control in the presence of acute seroproliferative glomerulonephritis. The renal glomeruli were enlarged because of proliferation of mesanginal cells and accumulation of Shumlyanskii's serous exudate in the mesangium and cavity of the capsule, accompanied by cells of desquamated epithelium in some glomeruli. Cloudy-swelling and, in some areas, balloon hydropic dystrophy developed in the convoluted tubules, accompanied by narrowing of the lumen of the tubules. In rats receiving renalin, acute productive glomerulonephritis also developed but was characterized by less marked proliferation of mesangial cells and absence of balloon hydropic dystrophy.

The results are thus evidence that renalin, an alkaline polypeptide from the kidney, can prevent the further development of Masugi nephritis.

LITERATURE CITED

1. V. P. Baluda, Z. S. Barkagan, and E. D. Gol'dberg, Laboratory Methods of Investigation of the Hemostasis System [in Russian], Tomsk (1980).
2. B. I. Kuznik and N. N. Tsybikov, Usp. Sovrem. Biol., 92, No. 5, 243 (1981).
3. Y. G. Morozov and V. Kh. Khavinson, Eksp. Khir., No. 2, 495 (1974).
4. V. G. Morozov and V. Kh. Khavinson, Usp. Sovrem. Biol., 96, No. 6, 339 (1983).
5. D. N. Novikov and V. I. Novikova, Cellular Methods of Immunodiagnosis [in Russian], Minsk (1979).
6. É. P. Chenchikova and I. N. Potapova, Byull. Éksp. Biol. Med., No. 10, 548 (1977).

SERUM AND PLASMA ERYTHROPOIETIC DETERMINATION IN MICE WITH PHENYLHYDRAZINE ANEMIA

T. E. Manakova
and N. A. Setkov

UDC 616.155.194-092.9-092:[616.154.61:577.175.85

KEY WORDS: Erythropoietin, mouse serum and plasma, anemia, method of testing.

Erythropoiesis in vivo is regulated with the participation of the plasma hormone erythropoietin [1]. The study of its properties and the mechanism of its action and synthesis in the body and determination of its concentration in biological fluids cannot be undertaken without the use of adequate methods of assessment of activity of the hormone. Many methods of analysis of erythropoietic activity have been described, but many of them suffer from various disadvantages: low sensitivity, laboriousness, the need to have large quantities of the erythropoietin standard, and sensitivity to impurities.

A micromethod requiring at least 5-10 times smaller doses of erythropoietin than the majority of methods hitherto used has recently been developed [9], and is based on recording incorporation of ^{3}H-thymidine into DNA of erythroid cells under the influence of erythropoietin. In the present investigation a modified method of Krystal [9] was used to determine erythropoietic activity of erythropoietin-enriched mouse serum and plasma. Since the use of any method requires an erythropoietin standard, availability of which is limited, we assessed the possibility of using the serum or plasma of a mouse with phenylhydrazine anemia as the source of erythropoietin, inducing equivalent stimulation of proliferative activity of erythroid cells.

EXPERIMENTAL METHOD

Female CBA and (CBA × $C_{57}BL)F_1$ mice weighing 20-30 g were used in the experiments. Anemia was induced by intraperitoneal injection of phenylhydrazine hydrochloride (PH) in isotonic solution neutralized with NaOH, on two successive days in a dose of 60 mg/kg (0.2-

Central Research Institute of Hematology and Blood Transfusion, Ministry of Health of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR O. K. Gavrilov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 1, pp. 21-24, January, 1989. Original article submitted November 4, 1987.